

## PSC 4-Marker Immunocytochemistry Kit

## PSC (OCT4, SSEA4) Immunocytochemistry Kit

## PSC (SOX2, TRA-1-60) Immunocytochemistry Kit

Catalog no. A24881, A25526, A25525

Table 1 Contents and storage

Kit component	Part no.	Concentration	Amount	Storage	Usage notes
<b>Primary antibodies</b>					
anti-OCT4 (host: rabbit)	A24867	100X	20 µL	-20°C to 4°C	Dilute with Blocking Solution
anti-SSEA4 (host: mouse IgG3)	A24866				
and/or					
anti-SOX2 (host: rat)	A24759				
anti-TRA-1-60 (host: mouse IgM)	A24868				
<b>Secondary antibodies</b>					
Alexa Fluor® 555 donkey anti-rabbit; for use with anti-OCT4	A24869	250X	20 µL	-20°C to 4°C; avoid freeze-thaw cycles	Ex/Em* 555/565 nm (orange); spin before use**
Alexa Fluor® 594 donkey anti-rabbit; for use with anti-OCT4	A24870				Ex/Em* 590/617 nm (red); spin before use**
Alexa Fluor® 488 goat anti-mouse IgG3; for use with anti-SSEA4	A24877				Ex/Em* 495/519 nm (green); spin before use**
and/or					
Alexa Fluor® 488 donkey anti-rat; for use with anti-SOX2	A24876				Ex/Em* 495/519 nm (green); spin before use**
Alexa Fluor® 555 goat anti-mouse IgM; for use with anti-TRA-1-60	A24871				Ex/Em* 555/565 nm (orange); spin before use**
Alexa Fluor® 594 goat anti-mouse IgM; for use with anti-TRA-1-60	A24872				Ex/Em* 590/617 nm (red); spin before use**
<b>Additional reagents</b>					
NucBlue® Fixed Cell Stain (DAPI nuclear DNA stain)	R37606	NA	1 vial	-20°C to ambient temperature	Ex/Em* 358/461 nm (blue); apply 1–2 drops/mL
Fixative Solution	A24344	1X	10 mL		4% formaldehyde in DPBS
Permeabilization Solution S	A24878		1% Saponin in DPBS		
Blocking Solution	A24353		3% BSA in DPBS		
Wash Buffer	A24348	10X	20 mL		10X DPBS, dilute to 1X with water†

**Handling and shelf life:** Use aseptic technique when handling all reagents. Allow frozen reagents to thaw completely before using them. Once thawed, do not re-freeze the kit (aliquots are not recommended). Store at 2°C to 8°C for up to 6 months.

\* Approximate excitation/emission wavelength maxima.

\*\* Centrifuge Secondary Antibody solutions (e.g., 2 minutes at 10,000 × g) and add only the supernatant to the Blocking Solution to minimize transferring protein aggregates that may have formed during storage, thereby reducing non-specific background staining.

† Upon thawing the 10X Wash Buffer, a precipitate may be observed that should go back into solution when warmed to ambient temperature and mixed well.

## Description

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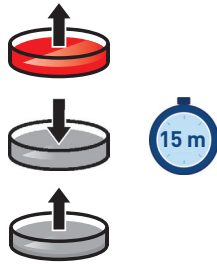
The PSC Immunocytochemistry Kits contain sets of primary and secondary antibodies along with ready-to-use buffers to enable convenient immunocytochemistry characterization of human pluripotent stem cells (hPSC). The primary antibodies included in these kits target well-established hPSC markers (OCT4, SOX2, SSEA4, and/or TRA-1-60) and were carefully selected to help ensure excellent performance in immunocytochemistry applications.

## Experimental protocol

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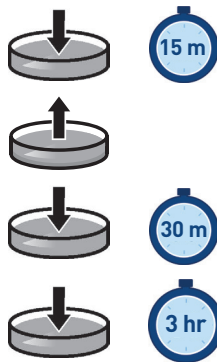
See Table 2, page 3, for recommended volumes. See Table 3, page 3, for multiplex staining options.

**Caution:** Use care when adding or removing liquids to minimize the possibility of dislodging the cells.



1. Remove media from the cells.
2. Add Fixative Solution and incubate for 15 minutes at room temperature.
3. Remove Fixative Solution.

*Optional stopping point:* After removing Fixative, add Wash Buffer (diluted to 1X with water), parafilm the sample to prevent it from drying out, and store at 4°C for up to 1 month.



4. Add Permeabilization Solution and incubate 15 minutes at room temperature.
5. Remove Permeabilization Solution.
6. Add Blocking Solution and incubate 30 minutes at room temperature.
7. Add desired primary antibody (see Table 3 for co-staining options) directly to the Blocking Solution covering the cells to yield a 1X final dilution, mix gently, and incubate for 3 hours at room temperature (or overnight at 4°C).



8. Remove the solution. Add Wash Buffer (diluted to 1X with water) and wait for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.



9. Add the appropriate Secondary Antibody (diluted to 1X in Blocking Solution; see Table 3 for guidance) and incubate for 1 hour at room temperature.



10. Remove the solution. Add Wash Buffer (diluted to 1X with water) and wait for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.

*Optional:* Add 1–2 drops/mL of NucBlue® Fixed Cell Stain (DAPI) into the last wash step and incubate for 5 minutes.



11. Image the cells immediately or store cells at 4°C in the dark, wrapped with parafilm to prevent the samples from drying out, for up to 1 month. Alternatively, for prolonged storage, apply a suitable antifade mounting medium, such as ProLong® Diamond Antifade Mountant, to the sample.

**Table 2** Recommended final volumes to use during the protocol.

Culture format	No. of tests*	Volume	Amount of each primary antibody to add	Amount of each secondary antibody to add
96-well plate	40	50 µL/well	0.5 µL	0.2 µL
48-well plate	20	100 µL/well	1 µL	0.4 µL
24-well plate	10	200 µL/well	2 µL	0.8 µL
12-well plate	5	400 µL/well	4 µL	1.6 µL
6-well plate	2	1000 µL/well	10 µL	4 µL
35-mm dish	2	1000 µL/dish	10 µL	4 µL
4-well chamber slide	5	400 µL/well	4 µL	1.6 µL
8-well chamber slide	10	200 µL/well	2 µL	0.8 µL

\* When using the suggested staining volume, this kit contains sufficient reagents for the indicated number of tests per primary antibody.

**Table 3** Dual antibody staining options. Note that the NucBlue® Fixed Cell Stain (a DAPI nuclear DNA stain) provided in this kit is also compatible with these antibody combinations. See Figure 1, page 4, for example pictures.

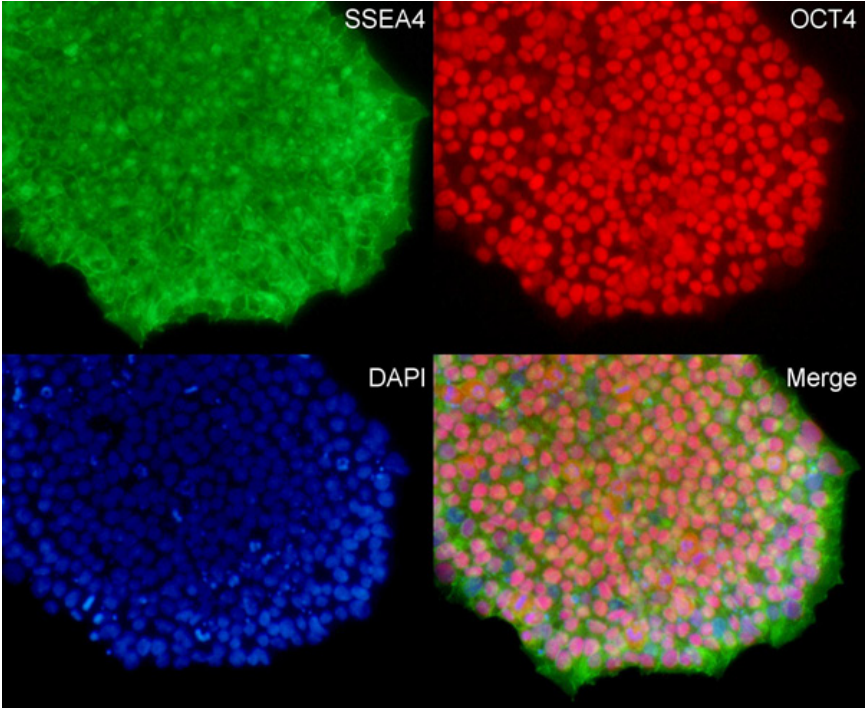
Color options:	Green* (e.g., FITC filter)	Orange* (e.g., Cy <sup>3</sup> / TRITC filter) or Red* (e.g., Texas Red® filter)
<b>Antibody combination # 1: SSEA4 + OCT4</b>		
Primary antibody	anti-SSEA4 (host: mouse IgG3)	anti-OCT4 (host: rabbit)
Secondary antibody	Alexa Fluor® 488 goat anti-mouse IgG3	Alexa Fluor® 555 donkey anti-rabbit or Alexa Fluor® 594 donkey anti-rabbit
<b>Antibody combination # 2: SOX2 + TRA-1-60</b>		
Primary antibody	anti-SOX2 (host: rat)	anti-TRA-1-60 (host: mouse IgM)
Secondary antibody	Alexa Fluor® 488 donkey anti-rat	Alexa Fluor® 555 goat anti-mouse IgM or Alexa Fluor® 594 goat anti-mouse IgM

\* See Table 1, page 1, for approximate excitation/emission wavelength maxima.

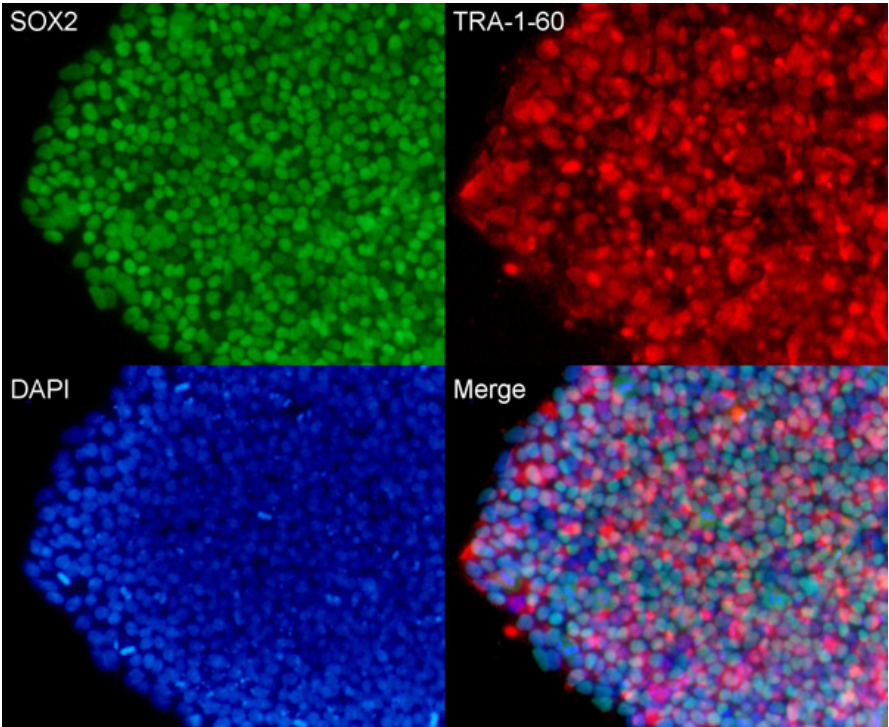
# Example data

Figure 1 Induced pluripotent stem cells (iPSC) were stained for pluripotent markers SSEA4 and OCT4 (antibody combination #1) or SOX2 and TRA-1-60 (antibody combination #2) and nuclear DNA (DAPI) using the PSC 4-Marker Immunocytochemistry Kit [Cat. no. A24881].

Antibody combination # 1: SSEA4 + OCT4 with additional DAPI (nuclear DNA) staining.



Antibody combination # 2: SOX2 + TRA-1-60 with additional DAPI (nuclear DNA) staining.



## Product list

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product name	Unit size
A24881	PSC 4-Marker Immunocytochemistry Kit	1 kit
A25526	PSC (OCT4, SSEA4) Immunocytochemistry Kit	1 kit
A25525	PSC (SOX2, TRA-1-60) Immunocytochemistry Kit	1 kit
<b>Related products</b>		
A25538	3-Germ Layer Immunocytochemistry Kit	1 kit
P36965	ProLong <sup>®</sup> Diamond Antifade Mountant	5 x 2 mL
A15871	TaqMan <sup>®</sup> hPSC Scorecard <sup>™</sup> Kit, FAST 96 well	2 plates
A14353	Alkaline Phosphatase Live Stain	50 µL
A18945	Gibco <sup>®</sup> Human Episomal iPSC Line	1 vial
A16517	CytoTune <sup>®</sup> -iPS 2.0 Sendai Reprogramming Kit	1 pack
A14703	Episomal iPSC Reprogramming Vectors	1 kit
A15960	Epi5 <sup>™</sup> Episomal iPSC Reprogramming Kit	1 kit
A1517001	Essential 8 <sup>®</sup> Medium	500 mL
A14700	Vitronectin (VTN-N) Recombinant Human Protein, Truncated	1 mL
A24354	Human Neural Stem Cell Immunocytochemistry Kit	20 tests
A1647801	PSC Neural Induction Medium	500 mL

## Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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